Invasive plant species and competition for pollinators

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Abstract

Invasive alien species cause harm to native flora and fauna, and can ultimately via biotic interactions and alterations of the abiotic conditions change entire ecosystems. They have therefore during the last decades gotten more attention and are now recognised as one of the major threats to biodiversity worldwide. When invasive plants establish in an area, one concern is that their presence can affect foraging patterns of pollinators. This can potentially have implications for pollination and reproduction of native plant species, and thus also for the local biodiversity. Here, I investigate this issue by studying how the invasive Japanese rose (*Rosa rugosa*) affects pollination, pollen loads on stigmas and seed set of the native common bugloss (*Anchusa officinalis*) in the coastal habitat of Lomma, Scania. During summer 2013 common bugloss plants in patches suffering from different degree of rose invasion were observed and compared. The results show that the two plant species share pollinators, and that the degree of rose invasion in a patch could alter the species composition and foraging behaviours of pollinators visiting the native plant. Different pollinators seem to respond differently to the rose. To understand such patterns the traits and ecology of the species should be considered. The number of visits to a certain plant was positively correlated with its number of flowers, and negatively correlated with the number of adjacent common bugloss flowers. Further, I found indications that the Japanese rose affected pollinator visitation to common bugloss flowers, proportion of correct pollen grains on bugloss stigmas and seed weight. Although no effects were seen on number of seeds, Japanese rose may, by impacting pollinator communities and competing for space and nutrients, impact a variety of native plant species and organisms interacting with those. I suggest that future research and conservation efforts regarding invasive plants use an ecosystem-based approach and include the aspect of complex plant-pollinator interactions.

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1. Introduction

Today, one of the biggest concerns for biodiversity is the spread of invasive alien species (Convention on Biological Diversity 2015). Invasive alien plants that successfully establish in new areas can impact the local ecosystem functioning, for example through competition with native plant species for space, water, nutrients and pollinators (Convention on Biological Diversity 2015). A lack of any of these resources can lead to reduced seed production and underdeveloped seed set, why such competition with invasive species can result in limited reproduction of native species. The presence of invasive plants presence can therefore alter important functions and interactions between organisms in an ecosystem, with negative consequences for both flora and fauna (Convention on Biological Diversity 2015).

As many wild plant species are dependent on wild pollinators for reproduction (Ollerton et al. 2011), competition for pollinators between native and invasive plant species can have implications for the long-term sustenance of native species. Invasive plants can affect the choice of food source and foraging behaviour of pollinators (Bjerknes et al. 2007), something that can reduce intraspecific pollen transfer, while simultaneously increasing interspecific pollen transfer (Morales & Traveset 2008). This can result in pollen limitation, i.e. when seed set and plant reproduction is limited by lack of pollen (Morales & Traveset 2008). The historical loss of pollinator species and trait diversity (Biesmeijer et al. 2006, Dupont et al. 2011) might further enhance the risks associated with competition between native and invasive plant species. In Sweden, both alien plant species and population trends in pollinator communities are recognised as big issues for wild plant communities (Environmental Objectives 2015, Jordbruksverket 2015). The combined effect of loss of pollinator diversity and the spread of invasive species is important to investigate, in order to understand how introduction of certain plant species, via pollinators, can affect not only the reproduction of native plants, but in the extension also entire ecosystems.

In this study, competition for pollinators between native and invasive species will be studied with the native species common bugloss (*Anchusa officinalis*) as target organism. It is found coexisting with the garden escapee Japanese rose (*Rosa rugosa*) in the coastal habitat of Lomma beach. The Japanese rose has intentionally been introduced in many parts of the world as a garden ornamental bush for its attractive flowers or for sand control purposes due to its soil stabilizing qualities (Bruun 2005). This has though not been free from negative consequences, since the rose thrives in certain areas where it has been introduced, and there has come to dominate the vegetation (Bruun 2005). Some of the consequences of the rose for the rest of local plant communities are easily recognised, since much of its success seems to spring from its vegetative reproduction and growth way. The species forms dense impenetrable stands of thorny braches and foliation (Bruun 2005), shadowing the soil, preventing other species from establishing and thereby outcompeting them. In addition to these well-known competitive skills of the rose, I will here investigate the more unknown effects of indirect interactions with native plants via pollinators.

The pollinators and their behaviours are therefore central for understanding how the two plants might influence each other. The foraging behaviour of pollinators controls pollen transfer between common bugloss flowers and hence also the seed production and reproduction. Heterospecific pollen transfer between flowers of different species can lead to clogging of stigmas, so that successful fertilization by conspecific pollen is hindered through for example inhibition of pollen tube growth (Morales & Traveset 2008). Further, the amount of conspecific pollen available can also be reduced if pollinators forage several different plant species simultaneously, something which also can reduce chances of fertilisation (Morales & Traveset 2008). Previous studies have shown that pollinator behaviour is affected by for example the floral offer in a specific area (Andersson 1984) and the abundance of invasive plants (Bjerknes et al. 2007). Thus, depending on the abundance of different kinds of flowers in the surrounding, a certain common bugloss flower might get more or less visits of higher or lower quality.

Further, for successful reproduction fertilisation must be followed by seed growth, something that requires resources like water and nutrients (Vaughton & Ramsey 1995). Invasive plants are often highly competitive regarding these resources (Convention on Biological Diversity 2015), and can thus once again be limiting reproduction of natives', although through other ways than competition for pollinators.

1.1 Aim of the study

Determine if and in that case how the invasive species Japanese rose affects pollination of the native species common bugloss, by comparing the pollinator visitations to, pollen deposition on and seed set of common bugloss plants in patches with different amount of Japanese rose.

1.2 Questions to answer

1. **Do the two species share pollinators?**

    a) What species visit common bugloss and Japanese rose respectively?

2. **Is pollinator visitation to common bugloss affected by presence of Japanese rose flowers?**

    a) Does the degree of rose invasion in a patch affect the number of flower visits to common bugloss?
    b) Does the number of rose flowers within 1 m affect the number of flower visits to common bugloss?
    c) Does the pollinator community composition differ among patches with different degree of rose invasion?

3. **Is the number of pollinator visits to common bugloss plants affected by the floral abundance of the species?**

    a) Is there a correlation between the number of visits to a certain plant and the number of flowers on that plant?
    b) Is there a correlation between the number of visits to a certain plant and the number of other common bugloss flowers within 1 m?

4. **Does the presence of the rose increase the risk of stigma clogging?**

    a) Is there a difference among patches of different degree of rose invasion regarding proportion of pollen from other species on stigmas of common bugloss plants?

5. **Is there a relationship between number of pollinator visitations and seed production in common bugloss?**

6. **Is there a relationship between the presence of Japanese rose and the seed production of common bugloss?**

    a) Does the number of seeds produced by a certain plant depend on the degree of rose invasion in the patch?
    b) Does the number of well-developed seeds produced by a certain plant depend on the degree of rose invasion in the patch?
    c) Does the weight of seeds depend on the degree of rose invasion in the patch?

2. Methods and Materials

2.1 Species description

3.1.1 Common bugloss (*Anchusa officinalis*)

The common bugloss is a quite common perennial in southern Sweden and is usually found on disturbed open land with sandy soil (Den virtuella floran 2015). It is a species native to Sweden and mentioned already in books from the Middle age (Den virtuella floran 2015). The first year it develops a rosette of leafs, the second it develops stems that separate into many branches with several drepanium inflorescences. One or two flowers per inflorescence bloom simultaneously, and when they begin to wither new flowers open up. The red-lilac-blue corollas are sympetalous with the petals fused into a tube around the stigma and stamens. White scales close the entrance to the tube, why relatively big and long-tongued pollinators like bumblebees are required for optimal pollination (Andersson 1984). Fertilisation requires pollen transfer between flowers via pollinators as the species is self-sterile (Andersson 1984). If pollination and other restricting factors are good, a maximum of four seeds per flower develop in a so-called schizocarp or "split-fruit" with four mericarps (seeds) (Den virtuella floran 2015).

3.1.2 Japanese rose (*Rosa rugosa*)

The Japanese rose (*Rosa rugosa*), native to Eastern Asia (Weidema 2006), was first introduced to Europe from Japan 1796, but mainly spread after late 1800s as the use as an ornamental bush increased (CABI 2013). It has thereafter naturalized and become common in some areas (CABI 2013, Herloff 2003). In Sweden, the first reports of naturalization are from 1918 (Milberg 1998). In parts of Europe, including regions of Sweden, the rose is regarded as one of the most troublesome aggressive invasive plant species (Milberg 1998). It is a member of the Rosaceae family, and the species name *rugosa* means wrinkly and refers to the appearance of the dark, richly nerved leaves (Weidema 2006). It is a shrub (1-2m height) with lots of branches covered in sharp thorns (Weidema 2006). Its rhizomes produce many shoots so that dense stands are formed, enabling outshadowing of other species and thereby domination of the habitat (Milberg 1998). The species reproduces not only sexually with seeds, but also vegetatively via the break-off of rhizomes and formation of clonal individuals (Bruun 2005, Weidema 2006). It flowers with big dark pink or white, fragrant flowers, offering plenty of pollen but no nectar (Bruun 2005). It is mainly cross-pollinated but self-compatible to some extent (Bruun 2005). In one Swedish study, Dobson et al. (1999) reported *Bombus terrestris* as the most common flower visitor.

2.2 Description of study site and focal plants

Fieldwork was conducted in June-August 2013 when both species were flowering. The study site was located in a small part of the northern shore of Lomma close to the mouth of Höje å. Three patches differing in degree of invasion by Japanese rose (amount and closeness to stands of the rose), but otherwise with similar vegetation were located. All patches had several individuals of common bugloss, and are described further in table 1.

Table 1. Descriptions of the three different patches investigated.

Degree of invasion	**Description**
High	Few common bugloss individuals growing sparsely between two big stands of Japanese rose
Low	Common bugloss growing without Japanese rose, but together with e.g. *Leymus arenarius*, *Symphoricarpos albus* and *Echium vulgare*
Moderate	Lots of common bugloss growing together with species like *S. albus*, *E. vulgare*, *Rubus sp.*, *Lamium album* and *Solanum dulcamara*, close to a big stand of Japanese rose

Five focal plants of common bugloss in each of the three patches were marked with numbered stripes and sticks so that they could be relocated later in the season. Focal plants of similar size were chosen in the different areas, so that the three groups would be comparable.

2.3 Pollinator visitations

I visited all focal plants three times throughout the season in order to observe pollinators. Each time, the closest surrounding of a focal plant was also investigated regarding abundance of Japanese rose flowers and of other common bugloss flowers, since this can affect pollinator behaviour and foraging strategies (refs). All flowers of the two species within the area of a circle with 1 m radius and the focal plant in the centre were counted. The presence of other flowering species was noted (table 1) but the exact number of flowers was not counted due to time restraints.

During 30 minutes, I then counted the number of pollinators visiting flowers of either a focal plant, other common bugloss plants and Japanese rose within the 1 m circle. If possible, I identified pollinators to species and noted the number of different flowers they visited. For practical reasons, smaller pollinators like beetles and flies were not included in the observation, but their importance for pollination of common bugloss is probably negligible (Andersson 1984).

To minimize differences between the patches due to sampling, I avoided doing observations of all focal plants in one patch the same day, by circulating through the patches. Focal plants of more or less the same size but in different patches were observed after each other in order to enhance comparability of the patches. This was important since pollinator activity is dependent on factors like weather and time of the day, generally being higher in warm windless days (Tuell & Isaacs 2010). Observations of pollinator visitations were done at temperatures of minimum 19°C and maximum 31°C. The wind situation varied between 1 and 4 on Beaufort's scale (Appendix A), when considering the strength of the wind during the 30 minutes, including strength and frequency of wind gusts.

2.4 Pollen load on stigmas

One stigma from each focal plant was collected in order to analyse pollen loads in laboratory. The collected stigmas were all put on a piece of a fuchsine colouring gel on microscope glasses. Each glass was marked so the origin of the stigmas could be remembered and then put on a laboratory hot plate. When the gel started to melt and liquefy the glass was removed and a cover glass was put atop the substance. Stagnation followed temperature drop and the edges of the cover glass was painted with transparent nail polish to enable storage of the samples.

The number of pollen grains in the samples was then counted in microscope. The grains on the stigma and in the surrounding colouring gel were noted. Common bugloss pollen is easily recognised due to its surface structures, but pollen from other species was pooled due to difficulties in species determination, why no separate analyse of Japanese rose pollen was made. The stigmas were chosen haphazardly for microscoping without me knowing the origin of the stigma, so that unintentional biases due to subconscious assumptions about focal plants in different areas were avoided.

In order to control that common bugloss pollen grains were correctly identified in the lab, I used hand-pollinated stigmas (in which only common bugloss pollen should be present) for comparison with the insect-pollinated stigmas.

2.5 Collection and assessment of seed sets

When the flowering season for the common bugloss population at the study site in Lomma was over, all seeds from the focal plants were collected and brought to the lab. There, the seed set of one focal plant at a time was studied by randomly choosing 100 flowers from a bag where all flowers from a single focal plant were mixed. I opened the fruit and counted the number of seeds (maximum 4), noted their condition (any deformations) and weighed them.

2.6 Statistical analysis

The statistical analyses were performed in R version 3.2.1 (The R Foundation for Statistical Computing 2015) using RStudio 0.99.489 (RStudio 2009-2015), with necessary packages installed. Three different types of models were used (table 2). Firstly, to check for differences among the three patches Kruskal-Wallis tests were used due to issues with heterogeneous variances and a small data set (table 2, questions 2a, 5-6). Secondly, to see if there were correlations between two parameters generalized linear models (glm, package stats (R Core Team 2015), test=GLM) were used (table 2, question 2b), with Quasipoisson distribution (due to overdispersion). However, as focal plants in a certain patch are not independent of each other, I in some cases needed to take the hierarchic experiment design into account by adding random factors to the model. Therefore in those cases I, thirdly, used generalized linear mixed models (glmer, package lme4 (Bates et al. 2015), test=GLMM), assuming Poisson distribution. In those cases the continuous independent variable was centred around 1 and standardized (table 2, questions 3a-b). I tested for overdispersion and corrected for it by using an observational level random effect when necessary (Bolker et al. 2011).

Table 2. Information about the data and tests used for the different questions. The choice of tests and probability distribution used is based on descriptive statistics, where data distribution and homogeneity of residuals were checked.

Question	Dependent variable	Independent variable	Test and probability distribution	Aggregation level	Random factor
2a	Number of pollinator visits to focal plants	Patch	Kruskal-Wallis	Total number per focal plant	-
2b	Number of pollinator visits to focal plants	Number of rose flowers within 1 m	GLM, Quasipoisson	Total number per focal plant	-
3a	Number of pollinator visits to focal plants	Number of flowers on focal plants	GLMM, Poisson	Total number per focal plant	Patch + Observational level random effect
3b	Number of pollinator visits to focal plants	Number of common bugloss flowers within 1 m	GLMM, Poisson	Total number per focal plant	Patch + Observational level random effect
4a	Number of seeds produced by focal plants	Number of pollinator visits to focal plants	GLMM, Poisson	Maximum number per focal plant	Patch + Observational level random effect
5	Proportion of pollen from other species on stigmas of focal plants	Patch	Kruskal-Wallis	Maximum per focal plant	-
6a	Number of seeds produced by focal plants	Patch	Kruskal-Wallis	Total number per focal plant	-
6b	Number of well-developed seeds produced by focal plants	Patch	Kruskal-Wallis	Total number per focal plant	-
6c	Weight of seeds produced by focal plants	Patch	Kruskal-Wallis	Mean seed weight per focal plant	-

3. Results

3.1 The pollinators

To see if the common bugloss and the Japanese rose share pollinators (question 1), the total number of visits by different pollinator species to the two plant species is presented in table 3. All pollinators observed visiting the rose were also seen visiting the common bugloss, and the three most common visitors to both plants were *Bombus terrestris*, *Bombus pascuorum* and *Bombus lapidarius*.

Table 3. The different pollinator species observed visiting the two plant species. In cases where species identification was difficult, pollinators where identified as precise as possible. "*Bombus* sp." represents observations of unidentified members of the *Bombus* genus, and "Unknown bee" represents observations of bees belonging to the clade *Anthophila*, but where genus was difficult to determine.

Plant species	Pollinator species	Number of visits
Anchusa officinalis	*Bombus hortorum*	781
	Bombus lapidarius	2241
	Bombus pascuorum	4549
	Bombus pratorum	3
	Bombus subterraneus	2095
	Bombus sylvarum	11
	Bombus terrestis	9859
	Bombus sp.	423
	Unknown bee	290
Rosa rugosa	*Bombus hortorum*	6
	Bombus lapidarius	41
	Bombus pascuorum	63
	Bombus subterraneus	1
	Bombus terrestris	211
	Bombus sp.	20
	Unknown bee	30

The composition of pollinator species visiting common bugloss (question 2c) differed among the three different patches (figure 1, for further information see Appendix A). In the patch suffering from the highest degree of rose invasion the dominating species was *B. pascuorum* (figure 1). However, the species was uncommon in the patch with a low degree of rose invasion (figure 1). In this patch, the dominating species instead was *B. terrestris* (figure 1), which also was a common species in the high degree patch. In the patch of moderate degree of rose invasion (figure 1) *B. terrestris* dominated while *B. pascuorum* was the second most common visitor.

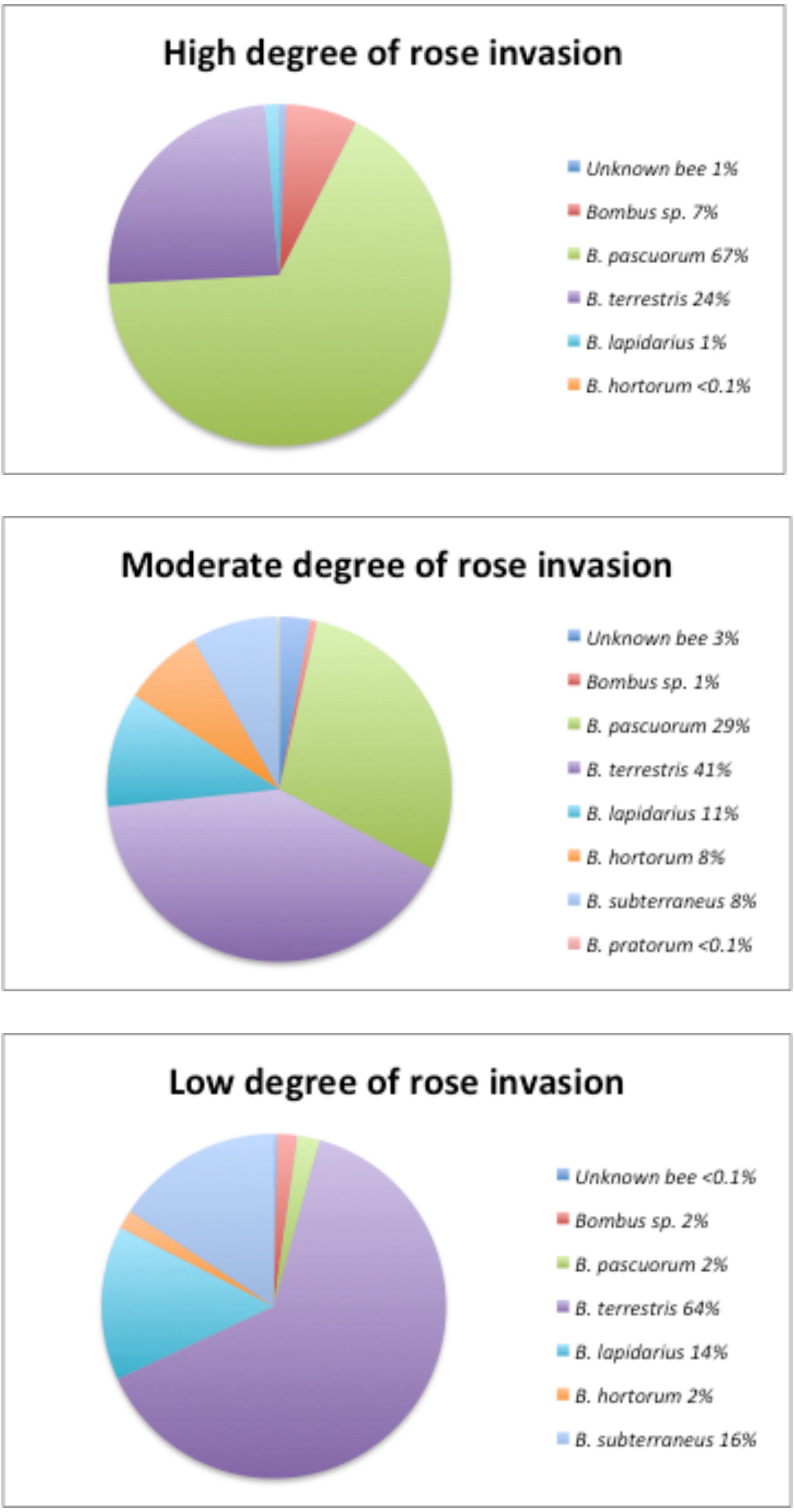

Figure 1. The proportion of visits different pollinator species did to common bugloss flowers in the three patches, suffering from a high, moderate or low degree of rose invasion.

3.2 Correlations

There was a statistically significant positive correlation between number of flowers on a focal plant (measured as standard deviation) and the number of visits that it gets (question 3a, table 4, figure 2). Model estimates showed that the number of visits to a plant increases with 215 % for each increase per standard deviation of the centred and standardized number of flowers on the plant. Contrary to this, the number of other adjacent common bugloss flowers showed a tendency (0.1>p>0.05) to negatively correlate with number of visits by pollinators to focal plants (question 3b, table 4, figure 3). For each increase in standard deviation of the centred and standardized number of common bugloss flowers nearby a certain plant, the number of pollinator visits to it decreases with approximately 51 %. The number of seeds produced also showed a tendency (0.1>p>0.05) to decrease with number of pollinator visits to focal plants (question 4a, table 4, figure 4). Model estimates show a 12 % decrease in number of seeds per increase in standard deviation of the centred and standardized number of visits.

Table 4. The results from correlation tests, p-values of significant (p<0.05) correlations in bold. Degrees of freedom (df) are presented for models based on glm. For glmer models no such values are provided, why the total number of observations (n) instead is reported.

Question	Dependant variable	Independent variable	Model	z-value	df or n	p-value
2b	Number of pollinator visits to focal plants	Number of nearby rose flowers	glm (Quasi-poisson)	0.35	14	0.73
3a	Number of pollinator visits to focal plants	Number of flowers on focal plant	glmer (Poisson)	5.79	15	**<0.001**
3b	Number of pollinator visits to focal plants	Number of nearby common bugloss flowers	glmer (Poisson)	-1.70	15	0.09
4a	Number of seeds produced by focal plants	Number of visits by pollinators to focal plant	glmer (Poisson)	-1.71	15	0.09

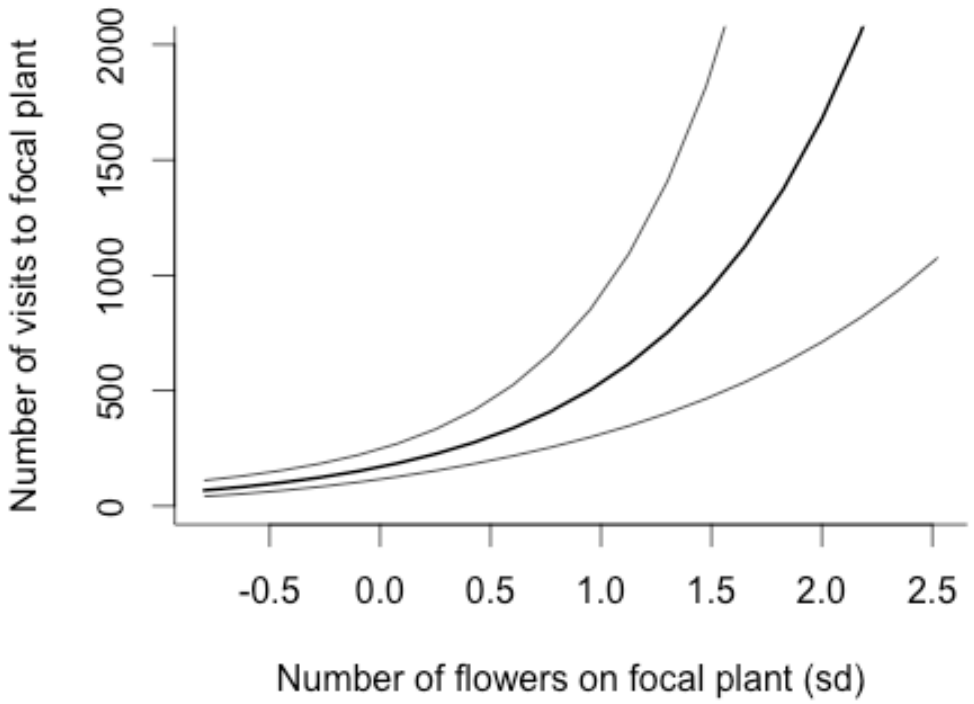

Figure 2. The positive correlation between centred and standardized number of focal flowers and the number of pollinator visitations a focal plant gets, answering question 3a. Outer lines represent 95% confidence interval as predicted by the statistical model.

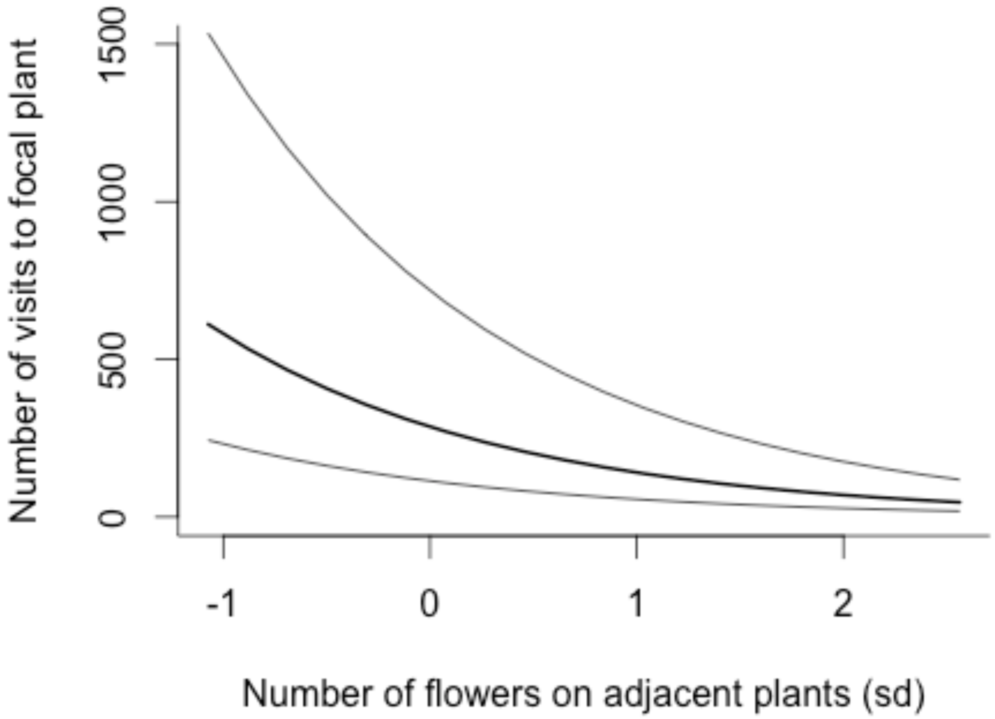

Figure 3. The negative tendency for correlation between centred and standardized number of nearby common bugloss flowers (within a circle) and the number of pollinator visitations a focal plant gets, answering question 3b. Outer lines represent 95% confidence interval as predicted by the statistical model.

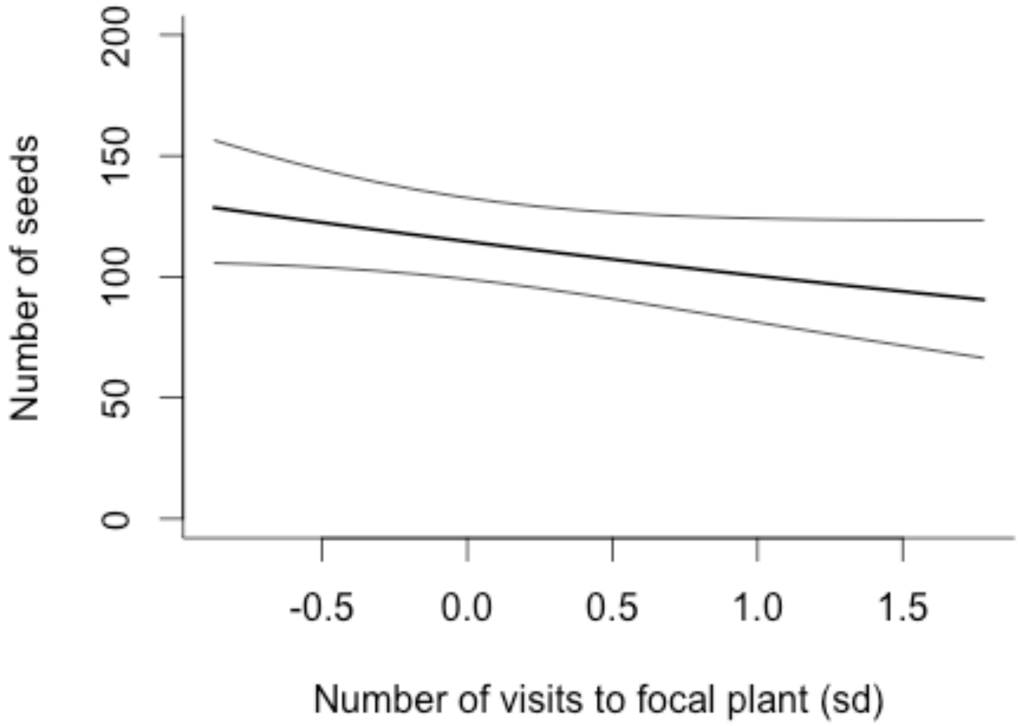

Figure 3. The negative tendency for correlation between centred and standardized number of pollinator visits to and the number of seeds a focal plant produces, answering question 4a. Outer lines represent 95% confidence interval as predicted by the statistical model.

3.3 Group comparisons

In table 5 the results from Kruskal-Wallis tests are presented. The only significant difference found is that seeds weigh more in the patch suffering from a moderate degree of rose invasion, than in the patch with a low degree of invasion (question 6c; figure 4). However, there are also tendencies ($0.1>p>0.05$) indicating differences among patches in some other cases. Regarding seed weight (question 6c), there are indications that seeds in the patch with low degree of rose invasion not only weighed less than seeds in the moderately rose invaded patch, but also less than in the patch heavily affected by rose.

Furthermore, comparisons both of number of visits by pollinators to focal plants (question 2a) and of the proportion of pollen from other species than common bugloss on stigmas (question 5) shows p-values closely above the limit for significance ($0.1>p>0.05$), thus indicating tendencies for potential differences among patches (table 5). Regarding the number of pollinator visits to focal plants (question 2a), there are indications that focal plants in the moderately rose affected patch are less frequently visited by pollinators than focal plants in both the highly and lowly affected patches. There are also indications that the proportion of pollen from other species than common bugloss on focal plant stigmas is higher in the two patches more affected by rose, compared to the patch with the lowest degree of rose invasion (question 5).

Table 5. The results from Kruskal-Wallis tests comparing the three different patches. Significant p-values ($p<0.05$) in bold.

Question	**Dependant variable**	**Compared patches**	χ^2	**Degrees of freedom**	**p-value**
2a	Number of visits by pollinators to focal plants	All	4.74	2	0.09
		High – Low	0.88	1	0.35
		High – Moderate	3.15	1	0.08
		Low – Moderate	3.15	1	0.08
5	Proportion of pollen from other species on stigmas of focal plants	All	4.10	2	0.13
		High – Low	2.98	1	0.08
		High – Moderate	0.10	1	0.75
		Low – Moderate	2.98	1	0.08
6a	Number of seeds produced by focal plants	All	2.66	2	0.26
		High – Low	2.47	1	0.12
		High - Moderate	0.10	1	0.75
		Low – Moderate	1.32	1	0.25
6b	Number of well-developed seeds produced by focal plants	All	2.80	2	0.25
		High – Low	2.15	1	0.14
		High – Moderate	0.10	1	0.75
		Low – Moderate	1.84	1	0.17
6c	Weight of seeds produced by focal plants	All	6.86	2	**0.03**
		High – Low	3.15	1	0.08
		High – Moderate	2.45	1	0.12
		Low – Moderate	4.81	1	**0.03**

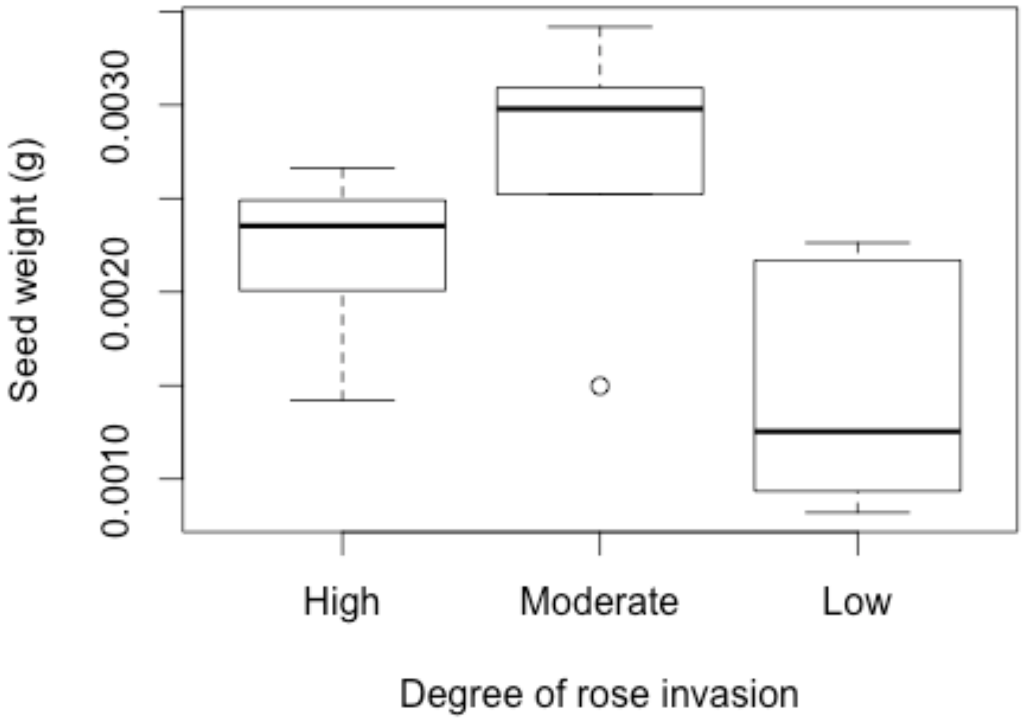

Figure 4. The weights in grams of seeds produced in the different patches (question 6c), significant difference between seed weight in the patches of low and moderate degree of invasion.

4. Discussion

My results show that common bugloss and Japanese rose are visited by the same pollinator species on Lomma beach, and thus that there possibly are plant interactions via pollinators. The dominating pollinator species visiting common bugloss varied among the three patches, why the degree of rose invasion in an area can be important, potentially by altering pollinator communities and/or foraging patterns. Something indicating this is the tendency to differences in pollen loads of common bugloss stigmas, with stigmas showing a higher proportion of pollen from other plant species in patches with high-moderate degree of rose invasion.

The number of pollinator visits to a certain common bugloss plant is positively correlated with the number of flowers on it, while an increasing number of flowers in the close surrounding instead seems to have a negative impact on the number of visits. Further, the number of seeds produced tends to negatively correlate with the number of pollinator visitations. The reason to this negative trend is not known, but if more visits leads to fewer seeds it could possibly be due to more visits somehow disturbs fertilisation, perhaps via a higher degree of heterospecific pollen transfer.

Common bugloss plants in a patch suffering from a moderate degree of rose invasion tends to be less frequently visited by pollinators, compared to plants in patches of high or low degree of invasion. The number of seeds produced shows a tendency to correlate negatively with the number of visits made by pollinators, but no differences can be seen among patches regarding number and development of seeds. The fact that there was a difference in number of visits but not in number of seeds produced among patches, indicates that seed development not only depends on competition for pollinators with Japanese roes, but also that the patches differ in other aspects than degree of rose invasion. This is further supported by differences in seed weight among patches. It is not unlikely that the different degrees of rose invasion not only affects pollinator foraging patterns, but also other environmental factors like water and nutrients restricting seed growth in common bugloss. The results of my study stresses that conservation of biodiversity must consider the potential for invasive species to compete for important a broad variety of resources.

Conservative choices of statistical methods for data analyses reduces the risks that any of the significant results found here are a result of random variations. It is though of high importance to point out that the results of this study only are applicable to the local situation on the study site Lomma. It might even be so that differences observed among patches not depend on the different degrees of invasion by the Japanese rose, but instead arise from other factors differing among the patches. To control that any effects seen on common bugloss in this study actually derive from competition with the rose, several other study sites needs to be included. My results do however show some correlations and differences between patches in this locality; the most interesting further discussed more in detail below.

4.1 The pollinators

The idea that the two plant species can compete for pollinators is confirmed by my results, since all pollinator species visiting the rose also visited the common bugloss. The three most common visitors to both plant species were all relatively common species with generalist foraging preferences, namely; *B. terrestris*, *B. pascuorum* and *B. lapidarius* (Dupont et al. 2011).

However, regarding other pollinator species there seems to be some differences in the plant preference patterns. For example was *B. subterraneus* observed as a relatively frequent visitor on common bugloss, at least compared to only visiting the rose once. It is a rare, long-tongued species (Dupont et al. 2011), probably preferring common bugloss above the rose and actively discriminating between the species while foraging. It has previously been argued that the probability of observing the true foraging preferences of rare species are small due to lack of observations, and that assumed dietary specialisations therefore are false (Goulson et al. 2005). The observed preference for common bugloss above Japanese rose for some pollinator species in this study could thus be the result of small population sizes. However, Goulson et al. (2005) concluded that with a small number of observations, rare species could be classified either as specialists or generalists, why the small dataset of my study should not be biasing the preferences of rare pollinators too much.

When it comes to the composition and proportion of pollinator species visiting common bugloss in the three patches, there are some interesting differences. *B. terrestris* and *B. pascuorum* are the most frequent visitors both in patches of high and moderate degree of rose invasion. The high abundance of both those species in the UK has been explained by them being generalists with broad dietary niches (Goulson et al. 2005). However in the patch with the lowest degree of invasion, *B. pascuorum* was a relatively uncommon species making only 2% of the total visits to common bugloss. Such differences among patches indicate that the presence of Japanese rose can have impacts on the foraging patterns of pollinators, and that different pollinator species respond differently to the presence of rose in a landscape.

Pollinator species that successfully utilize resources from rose flowers are attracted and therefore centred to stands of rose, why it is possible that patches without roses loose those pollinators. Most of those species do however not abandon native plant species completely, but some of them might chose to forage on native species only in the closest vicinity of rose stands. My results exemplify this with the data on *B. pascuorum* visitations; being a frequent visitor to common bugloss in patches of high or moderate degree of rose invasion, but being a rare visitor in a patch of low rose abundance.

This patchy occurrence of different pollinator species in a landscape (influenced by invasive alien plants) can have implications for long-term population stability in native species in several ways. Firstly, unless other pollinator species with similar traits and ecological function replace species that abandon areas where they once were common, native plant individuals far from the invasive plants might suffer from pollen limitation. Furthermore, even if pollinators are replaced, there could potentially be risks that a former plant population is split into smaller subpopulations with restricted genetic exchange, due to the patchy foraging patterns of pollinators caused by the rose. If some pollinators visit individuals close to rose while others visit individuals far from it, pollen transfer between those individuals will be limited. This could lead to inbreeding and make plant populations less resilient to changes in environmental conditions.

The rose may also indirectly affect native plant populations by altering pollinator communities in other ways. An indirect consequence of pollinators being unequally adapted to exploiting resources offered by the rose, is that some species will be more favoured by the presence of the rose than others. This can in turn lead to populations of some species being more healthy than others, due to the ample food supply they effectively use. If an area such as the beach in Lomma has a restricted amount of suitable nesting sites, and pollinator species that vary in their efficiency at exploiting the rose require similar nesting sites, pollinators that are more efficient rose foragers could potentially outcompete others for those. Further, the

Japanese rose could also alter the environment in which it grows by changing microclimate, soil conditions and plant communities (by direct competition for space), thereby changing the habitat so that certain pollinator species could be more favoured than others.

As indicated by my results, it is possible that the Japanese rose can alter the composition of pollinator communities in several different ways. This can have indirect consequences for other plant species like the common bugloss, if the rose "steals" or somehow disfavour the pollinators that usually pollinate those plants. On the other hand, the rose might also facilitate pollination of other plant species by supporting the pollinator community with pollen, so that the total numbers of pollinators increase (Bjerknes et al. 2007). However, this is probably mostly applicable to generalist pollinators, and not to more rare species with specialised plant-pollinator interactions (Bjerknes et al. 2007).

4.2 Intraspecific competition

The highly significant correlation between number of pollinator visits to and number of flowers on focal plants shown in this study is supported by several previous studies (e.g. Andersson 1984). Similarly, the nearly significant negative correlation between number of visits to focal plants and the number of nearby surrounding common bugloss flowers indicating intraspecific competition for pollinators is also supported by several other studies (e.g. Andersson 1984). These results show that the pollination of a certain plant is dependant on its own properties, but also on the surrounding environment in which it lives. This stresses the importance of studying how both intra- and interspecific plant interactions can affect pollination and reproduction in common bugloss. However, while more flowers on a certain plant individual attract more pollinators, visitation rate per flower and thus also number of seeds per flower decreases with increasing flower abundance (Andersson 1984). This results in a trade-off between attracting more pollinators and complete pollination of each flower. The intraspecific competition for pollinators is also somewhat necessary for pollen transfer between flowers to occur, enabling seed production.

4.3 Interspecific competition

Regarding interspecific competitions for pollinators, the results were not so clear as for intraspecific competition. There were no significant differences among patches of different degrees of rose invasion regarding number of visits by pollinators to common bugloss. As the two plant species share pollinators, this opposes the idea of interspecific competition. However, there was a tendency that common bugloss plants in the moderately rose invaded patch got fewer visits than plants both other patches. The expected difference among patches may be lacking in my results due to the small dataset, and not due to an actual lack of ecological differences. If it is so that common bugloss gets more visits both in areas of high and low abundance of rose compared to areas of moderate degree of rose invasion, this supports the speculation above about pollinators behaving patchily when foraging (section 5.1). Species preferring rose forage in rose rich areas, species avoiding rose dense areas (e.g. because they avoid competing pollinators or because they prefer other plants) forage in rose poor areas.

Another explanation to why the patch suffering from a moderate degree of rose invasion could be differing from the rest, highlights some methodological issues of this study – not only the degree of rose invasion was distinguishing the patches from each other. For example did the mentioned patch also have the most diverse flora, with many flowering species. To ensure that differences in number of visits by pollinators among patches actually derive from competition with the rose, studies of floral abundance and pollinator visits to all present plant species are needed. With this study design it was though already difficult to keep track of pollinators, and it would practically have been impossible for one person to notice all pollinators, their species and what plants they visited within a 1 m radius circle if all plant species were to be included.

Another result indicating the unequal conditions of the different patches is that the weight of seeds differed between patches. Seeds weighed significantly less in the patch to a low extent affected by rose compared to seeds in the moderately affected patch, and perhaps also less than seeds in the patch suffering from high degree of rose invasion (not significant). The reasons to this are difficult to explain, but it can be variations in for example wind exposure and soil conditions. The patch where seeds grew heavier was located relatively protected from wind why plants might be less stressed, while plants in the patch of low rose invasion were very exposed and also grew on a small hill. Even small topographic differences in a sand dominated soil with high permeability can lead to differences in soil moisture in the root zone. Further, plants have complex interactions with the soil, and the nutrients available for seed growth therefore could be differing among patches due to differences in local plant diversity. As Japanese rose could be altering soil conditions by dominating the habitat and thus consume lots of water and nutrients, the invasive species can indirectly affect native plants. The exact reasons for differences in seed weight are difficult to determine without direct measurements of soil moisture and nutrient levels.

My study further indicates that common bugloss stigmas have higher proportion of pollen from other species in patches of high and moderate degree of rose invasion, compared to the patch of low degree. However, potential differences in pollen loads and number of visitations among patches had no negative impact neither on the number of seeds produced or on their status when measuring number of deformations, why fertilisation in common bugloss within the scope of this study does not seem to be pollen limited. Bjerknes et al. (2007) do similarly as me in their review conclude that reproduction in natives not always is reduced, even though alien plants compete for pollinators. However, considering insecurities regarding status of pollinator communities due to for example effects of climate change, lack of suitable pollinators might limit reproduction of certain plant species in the future.

5. Conclusions

I here show that on Lomma beach, a landscape heavily affected and shaped by the invasive Japanese rose, pollination of and seed production in the native common bugloss to some extent differs among patches of different degree of rose invasion. It is therefore possible that the rose is competing with native species, however the reasons to the observed differences can also be differences among patches in other aspects. There is intraspecific competition for pollinators between common bugloss individuals, who also share pollinators with the rose. This enables interspecific interactions between the two plants via pollinators foraging both species. I show that the degree of rose invasion is important to consider, as well as the fact that different pollinator species have different functions in an ecosystem and can respond differently to presence of the Japanese rose. As there were no differences in number of seeds produced in the different patches, the seed production of common bugloss on Lomma beach does today not seem to be limited by fertilisation. In other words, pollen transfer between plants and/or competition for pollinators with the Japanese rose are not restricting common bugloss seed production. There are however several other ways that the rose can outcompete common bugloss and other native species, why further concern still is appropriate. Apart from the obvious competitive skills due to vegetative reproduction and formation of impenetrable stands, uncertainties regarding the impact the rose has on seed weight arose in this study. The potential of the Japanese rose to alter composition of pollinator communities and soil conditions, warrants further studies where measures of those parameters are improved, combined with inclusion of several other native plant species, more replicates of the different patches and repetitive measures over several seasons to account for variations among years.

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7. References

Anderberg A. 2008. Oxtunga *Anchusa officinalis* L. Den virtuella floran, Naturhistoriska riksmuseet. [http://linnaeus.nrm.se/flora/di/boragina/anchu/anchoff.html] Last visited 2015-11-13.

Andersson S. 1984. Pollinatorbeteendet som begränsande faktorer på fröproduktionen hos gullviva (*Primula veris*) och oxtunga (*Anchusa officinalis*). Institutionen för systematisk botanik, Lunds Universitet.

Bates D., Maechler M., Bolker B., Walker S. 2015. lme4: Linear mixed-effects models using

Eigen and S4_. R package version 1.1-9. https://CRAN.R-project.org/package=lme4 .

Bergman K.-O. 2009. Undersökningstyp: Linjeinventering av humlor. Version 1:0 2009-04-16. Handledning för miljöövervakning. Naturvårdsverket.

Biesmeijer J.C., Roberts S.P.M., Reemer M., Ohlemüller R., Edwards M., Peeters T., Schaffers A.P., Potts S.G., Kleukers R., Thomas C.D., Settele J., Kunin W.E. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*. Vol. 313, pp. 351-354.

Bjerknes A.-L., Totland Ø., Hegland S.J., Nielsen A. 2007. Do alien plant invasions really affect pollination success in native plant species? Biological conservation. Vol. 138, pp. 1-12.

Bolker B.M., Brooks M.E., Clark C.J., Geange S.W. Poulsen J.R. Henry, M., Stevens H., White J.-S.S. 2011. GLMMs in action: gene-by-environment interaction in total fruit production of wild populations of *Arabidopsis thaliana*. Revised version, part 1.

Bruun H.H. 2005. *Rosa rugosa* Thunb. ex Murray. *Journal of Ecology*. Vol. 93, pp. 441–470.

CABI. 2013. Rosa rugosa (rugosa rose). *Invasive Species Compendium*. Datasheet. [http://www.cabi.org/isc/datasheet/47835] Last visited 2015-12-07.

Carvalheiro L.G., Kunin W.E., Keil P., Aguirre-Gutiérrez J., Ellis W.N., Fox R., Groom Q., Hennekens S., Van Landuyt W., Maes D., Van de Meutter F., Michez D., Rasmont P., Ode B., Potts S.G., Reemer M., Roberts S.P.M., Schaminée J., WallisDeVries M.F., Biesmeijer J.C. 2013. Species richness declines and biotic homogenisation have slowed down for NW-European pollinators and plants. *Ecology letters*. pp. 1-9.

Convention on Biological Diversity. 2015. Invasive alien species – what's the problem? CBD Secretariat. [https://www.cbd.int/invasive/problem.shtml] Last visited 2015-12-22.

Den virtuella floran. 2015. Oxtunga Anchusa officinalis L. [http://linnaeus.nrm.se/flora/di/boragina/anchu/anchoff.html] Last visited 2015-12-22.

Dupont Y.L., Damgaard C., Simonsen V. 2011. Quantitative historical change in bumblebee (*Bombus* spp.) assemblages of red clover fields. *PLoS ONE*. Vol. 6. e25172.

Environmental Objectives. 2015. *Swedish Environmental Protection Agency*. [http://www.miljomal.se/Environmental-Objectives-Portal/] Last visited 2015-12-07.

Goulson D., Hanley M.E., Darvill B., Ellis J.S., Knight M.E. 2005. Causes of rarity in bumblebees. Biological conservation. Vol. 122, pp. 1-8.

Herloff B. 2003. Floraförändringar i Göteborgs södra skärgård under 1900-talet. *Svensk Botanisk Tidskrift*. Vol. 97, pp. 3-14.

Jordbruksverket. 2015. Humlor. *Jordbruksverket*. [https://www.jordbruksverket.se/amnesomraden/miljoklimat/ettriktodlingslandskap/mangfaldpaslatten/nyttodjur/humlor.4.37e9ac46144f41921cd150e9.html] Last visited 2015-12-15.

McNeely J.A., Mooney H.A, Neville L.E., Schei P., Waage J.K. (eds.). 2001. A global strategy on invasive alien species. *IUCN*. Gland, Switzerland, and Cambridge, UK.

Milberg P. 1998. Aggressiva invasionsarter. *Svensk Botanisk Tidskrift*. Vol. 92, pp. 313-321.

Morales C.L., Traveset A. 2008. Interspecific pollen transfer: magnitude, prevalence and consequences for plant fitness. *Critical reviews on plant sciences*. Vol. 27, pp. 221-238.

NOBANIS. 2015. Invasive Alien Species - Pathway Analysis and Horizon Scanning for Countries in Northern Europe. *Nordic Council of Ministers*. Copenhagen. Nordisk Ministerråd, pp. 222.

Ollerton J., Winfree R., Tarrant S. 2011. How many flowering plants are pollinated by animals? *Oikos*. Vol. 120, pp. 321–326.

R Core Team. 2015. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. [http://www.R-project.org/].

RStudio. 2009-2015. RStudio version 0.99.489. *RStudio Inc*.

Rasmont P., Pauly A., Terzo M., Patiny S., Michez D., Iserbyt S. Barbier Y., Haubruge E. 2005. The Survey of Wild Bees (Hymenoptera, Apoidea) in Belgium and France. Rapport d'activité, UMH - FUSAGx - FAO.

The R Foundation for Statistical Computing. 2015. R version 3.2.1.

Tuell J.K., Isaacs R. 2010. Weather during bloom affects pollination and yield of highbush blueberry. *J. Econ. Entomol.* Vol.103.

Vaughton G., Ramsey M. 1995. 17 Pollinators and seed set. In: Seed development and germination. Editors: Kigel J., Galili G. Marcel Dekker, New York.

Weidema I. 2006. NOBANIS – Invasive Alien Species Fact Sheet – *Rosa rugosa*. *European Network on Invasive Alien Species*.

Appendix A

Beaufort's wind scale

- 2 Beaufort: En vindflöjel (i gott skick) visar vindens riktning.
- 3 Beaufort: Vinden sträcker en vimpel, sätter blad och tunna kvistar i oavbruten rörelse.
- 4 Beaufort: Kvistar och tunna grenar rör sig. Damm och lössnö virvlar upp.
- 5 Beaufort: Mindre lövträd börjar svaja. Vågor med utpräglade kammar på större insjöar.
- 6 Beaufort: Stora trädgrenar sätts i rörelse. Det viner i telegraf- och telefontrådar (det är dock lite svårt att hitta några telegraftrådar idag).
- 7 Beaufort: Hela träd börjar svaja. Man går ej obehindrat mot vinden.
- 8 Beaufort: Kvistar bryts från träden och det börjar bli besvärligt att gå i det fria.
- 9 Beaufort: Mindre skador på hus. Rökhuvar och taktegel blåser ner.
- 10 Beaufort: Sällsynt i inlandet. Träd ryckas upp med roten, betydande skador på hus.

Reference: Swedish Metrological and Hydrological Institute. 2015. [http://www.smhi.se/kunskapsbanken/meteorologi/skalor-for-vindhastighet-1.252] Last visited 2015-12-22.

Table 1. The number of visits made by different pollinator species to common bugloss flowers (focal plant + other common bugloss plants within circles) in the three different patches, and the proportion of the total number of visits that the different species did in the three patches.

Patch	Pollinator species	Number of visits	Proportion of visits
A, high degree of invasion	*B. hortorum*	1	<0.01
	B. lapidarius	40	0.01
	B. pascuorum	1965	0.67
	B. terrestris	719	0.22
	Bombus sp.	199	0.07
	Unknown bee	20	0.01
B, low degree of invasion	*B. hortorum*	161	0.02
	B. lapidarius	1315	0.14
	B. pascuorum	191	0.02
	B. subterraneus	1426	0.16
	B. terrestris	5790	0.64
	Bombus sp.	170	0.02
	Unknown bee	32	<0.01
C, medium degree of invasion	*B. hortorum*	619	0.08
	B. lapidarius	886	0.11
	B. pascuorum	2393	0.29
	B. pratorum	3	<0.01
	B. subterraneus	669	0.08
	B. sylvarum	11	<0.01
	B. terrestris	3350	0.41
	Bombus sp.	54	0.01
	Unknown bee	238	0.03